Remarks:

Claims 3-4, 7-25, and 31 remain for consideration in this application with claims 12, 19, and 31 being in independent format. Applicant respectfully asserts that in view of the amendments and remarks herein, the rejections of the Office Action dated December 30, 2005 are traversed or should be withdrawn.

The Examiner rejected all claims as anticipated and obvious in view of the Scheffel reference. Scheffel describes a technique for determining chronic HCV infection by detecting a very specific antibody, namely that to the E2 protein of hepatitis C virus. In this method, a test sample suspected of containing anti-E2 antibody is contacted with antigen specific for the antibody, followed by detecting the amount of antibody present in the test sample, and correlating a high titer or a sustained titer with a diagnosis of chronic infection. In no case does Scheffel quantitate a plurality of different antibodies reactive with different antigens; his focus is entirely upon a single type of antibody, namely anti-E2 antibody. Scheffel also notes that a number of methods may be used to measure the concentration of antibody to E2, including optical density measurements.

The present invention is distinctly different from Scheffel. In the disclosed method, the samples undergoing testing are contacted with a multiple-antigen system in a first assay (c100-3, HC-31, and HC-34) which are reactive with different antibodies which may be in the samples. Thereafter, in preferred practice, a second assay is performed making use of three additional antigens (c22-3, c200, and NS5). The latter are a part of the ORTHO HCZ version 3.0 ELISA Test System. The protocols for this test system have been expressly incorporated by reference herein, and these instructions are attached as Exhibit A.

In short, in the initial step of the present invention, use is made of a plurality of different antigens reactive with different antibodies which may be present in the samples undergoing testing. Once this multiple-antigen assay is completed, the optical density of the resultant solution is taken as a predictor of chronic HCV infection. It has been found that the use of such multiple-antigen assays is an important feature in obtaining valid results with a minimum of false positives.

Nothing in Scheffel in any way suggests or intimates the present invention. In all instances, the solution being quantitated in Scheffel has in it *only* antigen reactive with E2 antibody. Scheffel mentions on page 17 that samples may be tested using a commercially available assay, but this system is not quantitated nor used for predictive purposes. Rather, such testing is employed to confirm that the sample comes from an individual who has been infected by HCV, but it is not quantitated or used for determining chronic infection.

In discussing the reference, the Examiner also asserted that "Scheffel teaches that optical density may be used to derive antibody concentration which is the variable that defines chronic HCV infection." It appears that Scheffel does assert that the concentration of a *specific* antibody, namely antibody to E2, is a defining variable for chronic infection variable.

However, it is equally true that the art recognizes that, in general, concentration of HCV antibodies is in no way predictive of chronic infection. Thus, attached as Exhibit B is a reprint from Zakim and Boyer's *Hepatology: A Treatment of Liver Diseases*, 5th Ed., which states:

> In sharp contrast to hepatitis B, the humeral immune response against HCV does not allow discrimination between different stages of infection (as for example with hepatitis B in which anti-HBcore IgM is indicative of acute HBV infection and anti-HBcore IgG is indicative of chronic or resolved HB infection). Antibodies against epitopes from all HCV proteins are detectable in acute as well as in chronic infection, and are also present after recovery from HCV. No

> specific antibody pattern is associated with recovery or with a specific level of replication.

Similarly, at page 673, attached as Exhibit C, the following appears:

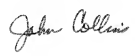
> The presence of anti-HCV indicates exposure to the virus, but does not differentiate between acute, persistent, or resolved infection. Antibodies against HCV persist in patients with spontaneously resolved infection, although titers decrease and may even disappear over time. Virological assays detect HCV RNA sequences, indicative of ongoing infection, and HCV RNA levels may fluctuate half a log even in the absence of therapy. Seriologic assays are typically used for screening and initial diagnosis, whereas HCV RNA assays are used in confirming infection and/or for monitoring treatment response.

The latter quote describes the status of the art at the time of the present invention, i.e., antibody assays were used to establish HCV infection, expensive and time-consuming HCV RNA assays were required to confirm chronic infection. However, the present invention overcomes this problem based upon the discovery that optical density values of samples contacted with multiple HCV antigens may be effectively correlated with known values to give valid predictions about chronic infection. The art nowhere suggests this concept, and indeed the art teaches away from it. The patentability of the present claims is therefore manifest.

If any questions should remain, the Examiner is encouraged to contact the undersigned at 1-800-445-3460. Any additional fee which is due in connection with this amendment should be applied against our Deposit Account No. 19-0522.

In view of the foregoing, a Notice of Allowance appears to be in order and such is courteously solicited.

Respectfully submitted,



Date: June 30, 2006

By

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816/474-9050

ATTORNEYS FOR APPLICANT

Hepatitis C Virus Encoded Antigen (Recombinant c22-3, c200 and NS5) *ORTHO® HCV Version 3.0 ELISA Test System*

Enzyme-Linked Immunosorbent Assay for the Detection of Antibody to Hepatitis C Virus (Anti-HCV) in Human Serum or Plasma

NAME AND INTENDED USE

ORTHO HCV Version 3.0 ELISA Test System is a qualitative, enzyme-linked, immunosorbent assay for the detection of antibody to hepatitis C virus (anti-HCV) in human serum or plasma.

SUMMARY AND EXPLANATION

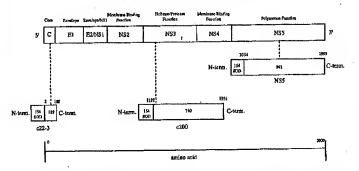
ORTHO HCV Version 3.0 ELISA Test System is an enzyme-linked immunosorbent assay (ELISA) which utilizes microwells coated with recombinant hepatitis C virus encoded antigens as the solid phase. ELISA technology utilizes the principle that antigens or antibodies which become bound to the solid phase can be detected by complementary antibody or antigen which is labeled with an enzyme capable of acting on a chromogenic substrate. When enzyme substrate is applied, the presence of antigen or antibody can be detected by the development of a colored end-product. Immunoassays of this type were first developed in the early 1970s.[1] Since that time, ELISA technology has been extensively used for the detection of antigens and antibodies for a wide range of infectious diseases.

The hepatitis C virus (HCV) is now known to be the causative agent for most, if not all, blood-borne non-A, non-B hepatitis (NANBH).[2-7] Studies throughout the world indicate that HCV is transmitted through contaminated blood and blood products, through blood transfusions or through other close, personal contacts. Currently, in the United States, greater than 90% of transfusion-associated hepatitis infections are considered to be NANBH infections.[8,9] Worldwide, other forms of NANBH are recognized.

Three recombinant hepatitis C virus encoded antigens are used in ORTHO HCV Version 3.0 ELISA Test System. The three recombinant antigens, developed by Chiron Corporation, are c22-3, c200 and NS5. A graphic representation of the putative HCV genome and recombinant proteins appears in Figure 1.

Figure 1

HCV Genome and Recombinant Proteins



HCV recombinant protein c22-3 is encoded by the putative core region of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that c22-3 is derived from a structural region of the genome which encodes the RNA-binding nucleocapsid protein. Nucleocapsid proteins are thought to be involved in forming the viral core structure. Recent studies have indicated that antibodies which develop following infection with HCV are often reactive with c22-3.[10] Moreover, studies performed using the CHIRON™ RIBA™ HCV 2.0 Strip Immunoblot Assay (SIA) for anti-HCV have shown that in many cases antibodies to c22-3 develop sooner following HCV infection than those to c100-3.[11]

HCV recombinant protein c200 is encoded by the putative NS3 and NS4 regions of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that c200 is derived from nonstructural regions of the genome. The c200 recombinant protein contains the c33c protein sequence genetically linked to the c100-3 protein sequence.

c33c is encoded by the putative NS3 portion of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that the NS3 region encodes the viral helicase, an enzyme involved in the unwinding of RNA during replication of the viral genome by RNA-dependent RNA polymerase. Recent studies have indicated that antibodies which develop following infection with HCV are frequently reactive with c33c. Studies performed using the CHIRON™ RIBA™ HCV 2.0 SIA for anti-HCV have shown that antibodies reactive with c33c often develop sooner following HCV infection than do those to c100-3.[11]

HCV recombinant protein c100-3 is encoded by the putative NS4 region of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that c100-3 is derived from a nonstructural region of the genome. At present, the function of this portion of the HCV genome is unknown. Antibodies which develop following infection with HCV are often reactive with c100-3.[4]

HCV recombinant protein NS5 is encoded by the putative NS5 region of the HCV genome. Amino acid and nucleotide sequence comparisons of flaviviruses and pestiviruses with HCV suggest that NS5 is derived from a nonstructural region of the genome that encodes the viral polymerase, an enzyme involved in replication of HCV. Recent studies have indicated that a significant proportion of persons infected with HCV develop antibodies to NS5.[5,15]

The use of HCV recombinant proteins derived from the core, NS3, NS4 and NS5 regions of the HCV genome has shown to be effective in identifying a greater number of diagnosed acute and chronic non-A, non-B hepatitis patients then single protein (c100-3) assays.[12,13] Therefore, the use of these additional proteins allows for earlier detection of seroconversion following HCV infection. Although antibody responses to NS5 region-encoded antigens are not as prevalent in response to HCV infection as those to core and NS3 region-encoded antigens, the addition of NS5 to c22-3 and c200 recombinant proteins in ORTHO HCV Version 3.0 ELISA Test System affords antibody detection to a greater number of HCV-encoded epitopes.

The amino acid sequence of the three HCV recombinant proteins is as follows.

Recombinant Protein	Polyprotein Sequence
c22-3	AA # 2-120
c200	AA # 1128-1931
NS5	AA # 2054-2995

The host organism for all three HCV recombinant proteins is *S. cerevisiea* (yeast).

The primary purpose of this assay is to screen blood donations so that units containing HCV antibody can be identified and eliminated from the blood supply. Although the presence of anti-HCV does not constitute a diagnosis of HCV infection, the determination of anti-HCV may be used as an aid in the diagnosis of hepatitis C and in the differential diagnosis of non-A, non-B hepatitis in conjunction with determination of liver enzymes, additional serological markers and clinical evaluation. The Hepatitis C Virus Encoded Antigen (Recombinant c22-3, c200 and NS5) used in the manufacture of ORTHO HCV Version 3.0 ELISA Test System is prepared under U.S. License by Chiron Corporation under a shared manufacturing arrangement.

PRINCIPLE OF THE PROCEDURE

The assay procedure is a three-stage test carried out in a microwell coated with a combination of recombinant hepatitis C virus (rHCV) antigen (c22-3, c200 and NS5).

In the first stage, a diluted test specimen is incubated in the test well for a specified length of time. If antibody reactive to any of the three antigens is present in the specimen, antigen-antibody complexes will be formed on the microwell surface. If anti-HCV is not present, complexes will not be formed. In the subsequent washing step, unbound serum or plasma proteins will be removed.

In the second stage, murine monoclonal antibody conjugated to horseradish peroxidase is added to the microwell. The conjugate binds specifically to the human IgG portion of the antigen-antibody complexes. If antigen-antibody complexes are not present, the unbound conjugate will be removed by subsequent washing.

In the third stage, an enzyme detection system composed of *o*-phenylenediamine (OPD) and hydrogen peroxide is added to the test well. If bound conjugate is present, the OPD will be oxidized, resulting in a colored end-product. In this reaction, peroxidase is divalently reduced by hydrogen peroxide to form an intermediate compound, which is, in turn, reduced to its initial state by subsequent interaction with hydrogen ion donating OPD. The resulting oxidized form of OPD has an orange color. Sulfuric acid is then added to stop the reaction.

The color intensity is dependent upon the amount of bound conjugate and therefore is a function of the concentration of anti-HCV present in the specimen. The color intensity is measured with a microwell reader (photometer) designed to measure light absorbance in a microwell.

REAGENTS

6X0 Test Kit Components (Product Code 930740)

5 Hepatitis C Virus (HCV) Encoded Antigen (Recombinant c22-3, c200 and NS5) -Coated Microwell Plates (96 wells each)-c22-3, c200 and NS5 derived from yeast

1 bottle Conjugate: Antibody to Human IgG (Murine Monoclonal) (125 mL)-anti-human IgG heavy chain (murine monoclonal) conjugated to horseradish peroxidase with bovine protein stabilizers
Preservative: 0.02% thimerosal

1 bottle Specimen Diluent (190 mL)-phosphate-buffered saline with bovine protein stabilizers
Preservative: 0.1% 2-chloroacetamide

1 vial OPD Tablets (30 tablets)-contains *o*-phenylenediamine•2HCl

1 bottle Substrate Buffer (190 mL)-citrate-phosphate buffer with 0.022% hydrogen peroxide
Preservative: 0.01% thimerosal

1 vial Positive Control (Human) (1.0 mL)
Source: Treated human serum or plasma containing anti-HCV and nonreactive for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). The anti-HCV serum or plasma has been treated to reduce the titer of potentially infectious virus. However, no test method can rule out the risk of potential infection; handle as if capable of transmitting infection.
Preservatives: 0.2% sodium azide and 0.9% EDTA

1 vial Negative Control (Human) (1.5 mL)
Source: Human serum or plasma nonreactive for HBsAg, antibody to HIV-1, antibody to HIV-2 and anti-HCV
Preservatives: 0.2% sodium azide and 0.9% EDTA

21 Plate sealers, disposable

3409 Test Kit Components (Product Code 930750)

25 Hepatitis C Virus (HCV) Encoded Antigen (Recombinant c22-3, c200 and NS5) -Coated Microwell Plates (96 wells each)-c22-3, c200 and NS5 derived from yeast

6 bottles Conjugate: Antibody to Human IgG (Murine Monoclonal) (125 mL each)-anti-human IgG heavy chain (murine monoclonal) conjugated to horseradish peroxidase with bovine protein stabilizers
Preservative: 0.02% thimerosal

4 bottles Specimen Diluent (190 mL each)-phosphate-buffered saline with bovine protein stabilizers
Preservative: 0.1% 2-chloroacetamide

5 vials OPD Tablets (30 tablets per vial)-contains *o*-phenylenediamine•2HCl

4 bottles Substrate Buffer (190 mL each)-citrate-phosphate buffer with 0.022% hydrogen peroxide
Preservatives: 0.01% thimerosal

4 vials Positive Control (Human) (1.0 mL each)
Source: Treated human serum or plasma containing anti-HCV and nonreactive for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2). The anti-HCV serum or plasma has been treated to reduce the titer of potentially infectious virus. However, no test method can rule out the risk of potential infection; handle as if capable of transmitting infection.
Preservatives: 0.2% sodium azide and 0.9% EDTA

5 vials Negative Control (Human) (1.5 mL each)
Source: Human serum or plasma nonreactive for HBsAg, antibody to HIV-1, antibody to HIV-2 and anti-HCV
Preservatives: 0.2% sodium azide and 0.9% EDTA

84 Plate sealers, disposable

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS AGENTS.

Store at 2 to 8°C

FOR IN VITRO DIAGNOSTIC USE

ORTHO HCV Version 3.0 ELISA Test System meets the FDA potency requirements.

PRECAUTIONS

1. CAUTION: Some components of this kit contain human blood derivatives. No known test method can offer complete assurances that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be handled as potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory practices.[14,16]
2. Wear disposable gloves while handling kit reagents and specimens. Thoroughly wash hands afterward.
3. All specimens should be handled as potentially infectious agents.
4. Sodium azide is included as a preservative in the Positive Control and Negative Control. Sodium azide has been reported to form lead or copper azides in laboratory plumbing. These azides are potentially explosive. To prevent build-up, flush plumbing with a large volume of water while disposing of these solutions in the sink. Following are the Risk and Safety Requirements.[16]
   R: 22 – Harmful if swallowed
   S: 28 – After contact with skin, wash immediately with plenty of water.
5. Handle and dispose of all specimens and materials used to perform the test as if they contain infectious agents. Disposal of all specimens and materials should comply with all local, state and federal waste disposal requirements.[17,18]
6. 4N sulfuric acid (H_2SO_4) is a strong acid. Wipe up spills immediately. Flush the area of the spill with water. If the acid contacts the skin or eyes, flush with copious amounts of water and seek medical attention.
7. Handle OPD tablets with plastic or Teflon®-coated forceps only. Metal forceps may react with tablets and interfere with the test results.
8. Avoid contact of OPD with eyes, skin or clothing, as OPD may cause irritation or an allergic skin reaction. If OPD should come into contact with the skin, wash thoroughly with water. OPD is toxic for inhalation, ingestion and skin contact. In case of malaise, call a physician. The following are the Risk and Safety Phrase Requirements.[16]
   R: 20/21-25-36-40-43 – Harmful by inhalation and in contact with skin. Toxic if swallowed. Irritating to eyes. Possible risks of irreversible effects. May cause sensitization by skin contact.
   S: 28-36/37/45 – After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
9. OPD tablets are light- and moisture-sensitive. Keep vial tightly closed when not in use. Bring vial to room temperature (15 to 30°C) before opening. The desiccant pouch must be retained in the vial at all times. Do not use tablets which are yellow or broken.
10. Distilled or deionized water must be used for Wash Buffer preparation. Clinical laboratory reagent water Type I or Type II is acceptable.[19] Store the water in nonmetallic containers.
11. Do not mix reagents from kits with different lot numbers. Any lot number of 20X Wash Buffer Concentrate may be used provided it is not used beyond its labeled expiration date.
12. All reagents and components must be at room temperature prior to use and kit components returned to 2 to 8°C after use.
13. The microwell strips are sealed in protective pouches with a humidity indicator desiccant. The desiccant, normally blue/purple in color, will turn pink if moisture is present in the pouch. If the desiccant is pink, the microwell strips should not be used.
14. Do not use reagents beyond their labeled expiration date.
15. Cross-contamination between reagents will invalidate the test results. Labeled, dedicated reservoirs for the appropriate reagents are recommended.
16. Ensure that specimen is added to the microwell. Failure to add specimen may produce an erroneous nonreactive result.
17. When using a single-channel micropipette for manual sample addition, use a new pipette tip for each specimen to be assayed. When using a multichannel micropipette, new tips are to be used for each reagent to be added.

IMMUNOPATHOGENESIS (see Chapter 8)

Since HCV is a non-cytopathic virus in most circumstances, it is the immune response rather than the virus itself that is central to the pathogenesis of liver disease. The immune response is also critical to clearance of virus following acute infection. For example, symptomatic patients with acute HCV infection are more likely to recover than asymptomatic patients.[54,55] Since symptoms are likely caused by the host's immune response, a strong cellular immune response appears to be key to viral clearance. Anti-HCV antibodies usually develop between months 2 and 3 of acute HCV infection, a time course that is late compared to other viral infections. The immune response against HCV is complex and generated by various cell types and tissues. Early innate immune responses may play an important role in determining the outcome of infection. The analysis of gene expression profiles in liver biopsies from chimpanzees during early HCV infection shows a very early increase of interferon-response genes, preceding expression of T-lymphocyte surface markers by several weeks. However, HCV has developed several mechanisms to inhibit innate responses, such as direct inhibition of natural killer (NK) cells by HCV envelope proteins via binding to CD81[56] or indirect impairment of NK-cell cytotoxicity by up-regulation of major histocompatibility complex class I molecules on infected cells.[57] Immune mechanisms play a role in the pathogenesis and progression of liver injury, since patients with more severe hepatitis have a higher chance of developing liver cirrhosis and HCC than those with less inflammation. The histological activity of the liver disease is determined by qualitative and quantitative assessment of the cellular infiltrate in the liver. This infiltrate consists mainly of T cells, NK cells, and NKT cells, thus representing an immune response with resulting "hepatitis."

In sharp contrast to hepatitis B, the humoral immune response against HCV does not allow discrimination between different stages of infection (as for example with hepatitis B in which anti-HBcore IgM is indicative of acute HBV infection and anti-HBcore IgG is indicative of chronic or resolved HBV infection). Antibodies against epitopes from all HCV proteins are detectable in acute as well as in chronic infection, and are also present after recovery from HCV. No specific antibody pattern is associated with recovery or with a specific level of replication. An early antibody response against the hypervariable region of the E2 protein (HVR-1) has been associated with a self-limited course of infection. Since there is high variability of the virus in this region, it seems possible that escape from efficient humoral immunity might occur with prolonged periods of viremia. Subsequently, a more heterogeneous humoral immunity against HVR-1 has been associated with viral persistence.[58] There seems to be no long-lasting protective humoral immunity against HCV. Anti-HCV antibody titers do decline after recovery from acute HCV infection and may become undetectable after two decades.[59] Thus, the prevalence of individuals who have had contact with HCV might be underestimated in the general population by anti-HCV testing alone, since anti-HCV may be negative in those with previous but resolved infection.

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Adaptive cellular immune responses are induced by dendritic cells (DC) that present antigens to CD4+ and CD8+ T cells. There is some evidence that DC function is altered by HCV,[60] although conflicting data have been published in recent years.[61] Nevertheless, there is a clear association between a multispecific, strong, and maintained HCV-specific CD4+ and CD8+ T-cell response and viral clearance during acute HCV infection.[62] The CD4+ response is maintained for several years after recovery. The CD8+ response also remains detectable, but there are conflicting data as to the extent that the CD8+ response decreases over time following recovery. Not only the frequency but, more importantly, the function of T cells determines the outcome of infection. Thus, resolution of HCV has been associated with an early interferon-gamma response by CD8+ T cells[63] while functionally impaired CD8+ T cells lead to viral persistence.[64] Hyporesponsiveness of T cells may be caused by immunosuppressive functions of HCV proteins. The balance between type 1 (such as interferon-gamma) and type 2 (such as interleukin-4 (IL-4) and IL-5) cytokines secreted by CD4+ and CD8+ T cells seems to be altered in chronic hepatitis C, an observation that may have relevance to HCV antiviral therapy. One proposed mode of action of ribavirin has been to shift the cellular immune response to a type 1-dominated immune response.[65] Activation of an immune response may also be a novel approach for HCV therapy. Early clinical trials of peptide or protein vaccination have already been performed, although it will likely be some time before therapeutic vaccination becomes part of standard therapeutic regimens for chronic hepatitis C.

Content

EXHIBIT C

DIAGNOSIS AND TESTING

DIAGNOSIS

Anti-HCV testing is accurate for making the diagnosis of infection in high-risk populations such as injection drug users, but may be negative in immune-compromised populations with HCV infection such as those with HIV, those on hemodialysis, or those following solid organ transplantation, and may be falsely positive in low-risk populations such as blood donors. The presence of anti-HCV indicates exposure to the virus, but does not differentiate between acute, persistent, or resolved infection. Antibodies against HCV persist in patients with spontaneously resolved infection, although titers decrease and may even disappear over time. Virological assays detect HCV RNA sequences, indicative of ongoing infection, and HCV RNA levels may fluctuate half a log even in the absence of therapy. Serologic assays are typically used for screening and initial diagnosis, whereas HCV RNA assays are used for confirming infection and/or for monitoring treatment response.[22,68,67]

SEROLOGICAL ASSAYS

The enzyme immunoassay (EIA) assays detect antibodies against different HCV antigens from the core and non-structural proteins. Serologic assays were first introduced in blood banks to screen donors in 1990, and were improved in 1992. Three generations of EIAs have been developed with increasing sensitivity and progressive decrease in the window period for seroconversion after acute exposure. Since the introduction of serologic assays for screening of donors, the risk of acquiring HCV infection from blood products has declined. The latest third-generation EIAs detect mixed antibodies against HCV core, NS3, NS4, and NS5 antigens, as soon as 7-8 weeks postinfection, with 99% specificity and sensitivity. Recombinant immunoblot assays (RIBA), while frequently used in the past for confirmation of true HCV exposure, have largely been replaced by sensitive virological assays, in which the absence of viral RNA is suggestive of resolved infection.

HCV RNA ASSAYS

HCV RNA can be measured by highly sensitive qualitative and quantitative assays.[22] Qualitative assays provide information about the presence or absence of virus and are generally more sensitive than quantitative assays. Qualitative HCV RNA detection may be accomplished by target amplification methods such as polymerase chain reaction (PCR) amplification or transcription-mediated amplification (TMA). Qualitative PCR detects as few as 50 IU/ml, while TMA has a sensitivity of 10 IU/ml. Specificity is 99% with both tests. Qualitative testing is largely used for confirmation of clearance of virus after apparently successful antiviral therapy or for the detection of virus in HCV-seropositive patients with chronic liver disease who lack detectable HCV RNA by quantitative assays. Other clinical situations where either qualitative or quantitative assays may be used include seronegative acute or chronic hepatitis in immunosuppressed patients, and the diagnosis of HCV infection in babies born to HCV-infected mothers. Most anti-HCV-positive patients with infection will have virus detectable by both qualitative and quantitative assays, since HCV RNA levels typically range between 5×10^4 and 5×10^6 IU/ml. US Food and Drug Administration (FDA)-approved tests for qualitative HCV RNA detection include the Amplicor HCV test v2.0 and the Cobas Amplicor HCV test v2.0, both with sensitivities of 50 IU/ml.

Qualitative HCV RNA assays (nucleic acid testing) are increasingly being used to test for low-level HCV RNA in blood donors with "serosilent" infection or in acutely infected donors in the "window" period before seroconversion. One in 230 000 donations can be identified to be HCV RNA-positive using nucleic acid testing.[68] These donors may transmit infection that may remain "serosilent" in the recipient. For this reason, many blood banks now routinely screen blood with nucleic acid tests, reducing the risk of transfusion-associated HCV infection to as low as 1:2 000 000 units transfused.[68]

Quantitative assays are useful in monitoring antiviral therapy, particularly 4 and 12 weeks after starting treatment. Patients who lack detectable HCV RNA (by either qualitative or sensitive quantitative assays) at 4 weeks into antiviral therapy are defined as having a rapid virological response (RVR); those who either lack HCV RNA *or* who have a two-log reduction from baseline values are defined as having an early virological response (EVR). Both these measures are increasingly being used to predict the likelihood of achieving sustained virological response (SVR) with therapy and/or to guide the duration of treatment.

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page 674

Methods to quantify HCV RNA levels in serum include signal and target amplification. The bDNA assay, commercially available through Bayer Diagnostics, is an example of signal amplification, that uses capture and target probes from the conserved 5' UTR and core regions of the virus to detect viral RNA. The amount of bound

probe is amplified through a series of synthetic branched DNA oligonucleotides. In target amplification techniques, HCV target RNA is reverse-transcribed and amplified using primers to the conserved 5' region of the HCV genome and the amount of viral RNA present in the amplified sample is estimated from a standardized dilutional series. HCV RNA levels are typically expressed as international units per milliliter and conversion factors have been derived to calculate IU values from copies for commonly used commercial assays (1 IU/ml corresponds to 0.9 copies/ml in the Amplicor HCV Monitor v2.0, 2.7 copies/ml in the Cobas Amplicor HCV Monitor v2.0, 3.4 copies/ml in the SuperQuant, 3.8 copies/ml in the LCx HCV RNA quantitative assay, and 5.2 copies/ml in the Versant HCV RNA 3.0 quantitative assay, respectively). Different commercial assays vary in their dynamic range. The lower limit of detection with current assays is approximately 600 IU/ml, while the upper end ranges from >500 000 IU/ml to 1 470 000 IU/ml. The Cobas Amplicor HCV Monitor v2.0 is an automated version of the Amplicor test and has a dynamic range of 600-500 000 IU/ml. Samples above the upper limit should be retested after dilution, particularly in those with high levels of virus prior to therapy, in whom EVR and RVR are going to be measured.

There are two commercially available assays for determining HCV genotype, assays based on PCR amplification of the 5' non-coding region. With these assays, the six genotypes can be readily identified, although tests are less accurate in measuring HCV subtypes, with errors occurring in 10-25% of cases because of variations in the target 5' NC region.

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